Control of a Tent Caterpillar, *Malacosoma incurvum discoloratum*, with a Commercial Formulation of *Bacillus thuringiensis* var. *thuringiensis* in Zion National Park, 1965

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INTRODUCTION

A tent caterpillar, *Malacosoma incurvum discoloratum*, has reached epidemic proportions in Zion National Park. In the Park, Fremont poplar (*Populus Fremontii*) is the principal host, and occasional feeding occurs on species of willow, cherry, apricot, and apple when they are present. Ash and sumac are not attacked.

Malacosoma incurvum is a voracious feeder and during its relatively short larval period (approximately 4 weeks) heavy populations can completely defoliate entire trees. The larvae consume practically the entire leaf, leaving only the midrib and portions of the lateral veins. Later in the summer after the insects have completed their feeding and pupated, the trees will refoliate, but consecutive attacks will cause branch killing, and sometimes, tree death. Fremont poplar has high aesthetic value as well as being the principal shade tree for picnic areas, campgrounds, and other heavily used recreational areas. Consideration of these factors together with extremely high tent caterpillar populations necessitated prompt control action.

BACKGROUND

Bacillus thuringiensis, var. *thuringiensis*, a commercially produced crystalliferous, spore-forming bacterium was the insecticide used. The product, Thuricide 90TS 1/, is an emulsifiable liquid containing approximately 30 x 10^9 (30 billion) viable spores per milliliter. A powdered formulation of Bt (Thuricide 60W 1/) was used to control the tent caterpillar in the Park in 1962, but control effectiveness was not fully assessed. In addition, the mechanical properties of this early powdered formulation were unsatisfactory for once mixed with water or other diluents it frequently clogged the spray system. 2/

1/ Registered trade mark, Bioferm Corp., Wasco, California

2/ Lewis, F. B., D. P. Connola and J. L. McDonough, 1962. Aerial Application of a *Bacillus thuringiensis* spore-crystal Concentrate in Fuel Oil and Water Formulation for Gypsy Moth Control - New York State, 1962. U.S. Forest Service. Northeastern Forest Experiment Station, Special Report (processed) 15 pp.

Noticeable settling occurred less than one minute after agitations, although it was difficult to distinguish a definite zone between the supernatant and precipitate. By one hour, however, the top of the precipitate was discernible at the 5 ml. mark in the bottom of the cylinder. Within two hours, settling was almost complete. The formulation was then resuspended with very little agitation.

This test showed that 90TS, mixed in the proportion designed for this project, should be well agitated immediately prior to use and subjected to some degree of agitation during use. The initial mixing can be accomplished by hand or by a recirculating pump system, then kept in suspension during use by normal pump vibration.

A test was run to determine the effects of extended dilution on the viability of the Bt concentrate over a reasonable length of time. This can be a problem in the field when the concentrate is mixed with water or other diluents and because of unfavorable weather or other unforeseen setbacks, the finished mix is allowed to set for an indefinite period before it is applied.

Spore viability counts were run over a nine day period using the same water (pH 8.0) used during the actual operation. The concentrate was diluted in the same proportion (1:20) used during the operation and held in a clear glass dilution bottle at room temperature for nine days.

At each plating, one ml. aliquots were pipeted from this initial dilution, diluted 10^{-8}, pour plated on tryptose agar, incubated for 24 hours at room temperature and colony counts made. As before, each plating was replicated three times. A total of 6 platings were made over the nine-day period.

Table I in the appendix shows the viability of diluted 90TS over an extended period. From this table it can be seen that the spore counts remained rather consistent during the nine-day period, except for the low count of 18.1×10^9 on the fourth day. Considering the enormous capability of human error in performing these intricate and precise dilutions, a variation of this magnitude is entirely normal. Nevertheless, the overall counts remained high and there is little evidence to support the suspicion that the endospores lose their viability once diluted with water and held in a mixed state for short periods of time. From this test it seems reasonable that once mixed with water (pH 8.0 or less), 90TS can be held under moderate temperatures for a few days without a significant loss of spore viability. However, once mixed and held for an indefinite period, then applied in the field and the diluents evaporate, the life span of the unprotected spore in unknown. The effect of extended dilution on the toxic action of the crystals and exotoxins is also unknown.

Area Treated

Approximately 1,000 acres of Fremont poplar were treated. This area included all Fremont poplars in the park that were within operational range of the mistblower: bordering the main highway, in campgrounds and picnic areas, near the residential compound and the Administration Building and in other public-use areas.

As a supplemental check to the final evaluation, a cursory analysis was made of dead larvae to determine whether mortality was caused by Bt. Periodic collections of ten dead larvae were taken from both sprayed and unsprayed areas following treatment. The first collection was made on April 30, two days after the initiation of treatment, and subsequent collections were made at two and three day intervals until May 12. Of the ten larvae collected, five were preserved in 70 percent alcohol and five placed dry in metal pill boxes. The individual collections were then mailed to Ogden for analysis. The interval between collection and examination averaged approximately 36 hours. Once received, the larvae were surfaced sterilized in 5% Clorox solution, mascerated, smeared on glass slides, heat fixed, stained with malachite green and counterstained with safranin red. Examination was made using a compound microscope with an oil immersion lens (970X). Determination of Bt kill was made on the basis of a reasonable number of bacillus present in each field.

RESULTS

Overall control results were judged as good. In the spray areas, for those trees that were in reach of the mistblower and were judged as receiving adequate coverage, protection of the foliage and kill of the tent caterpillar was excellent. In the upper crowns of the taller trees, that were beyond effective mistblower range, some defoliation occurred and a few residual tents were evident. Most of these tents, however, were considerably smaller than those tents in the check trees, and it is doubtful if they contained the same proportions of survivors.

In those trees close to roads but exceeding 50 feet in height or shadowed by other trees, control was variable.

All trees in the check areas exhibited some degree of defoliation. In some areas where pre-spray populations were exceptionally heavy, total defoliation occurred. These trees were covered with numerous large tents containing live, extremely active colonies.

The results of the investigation as to the cause of mortality is shown in Table 1 in the Appendix. Upon examination of this table, two factors become evident: first, all the dry preserved larvae from the sprayed areas contained sufficient bacilli to have caused death, and secondly, no bacilli were found in larvae taken from the check areas, regardless of the preservation method. In the spray area, vegetative cells were more numerous and sporulation more advanced in the dry preserved larvae than in those larvae preserved in alcohol. Evidently, germination and subsequent resporulation cease as soon as the larvae is killed by alcohol.

Bt killed larvae were found two days after treatment. The dead larvae were shrunken, flacid and black in appearance. In some of the treated areas, literally thousands of dead larvae were observed hanging from tents and branches or covering the ground. In less than 24 hours following treatment, infected larvae ceased feeding and took on a moribund appearance.

APPENDIX

TABLE I

Spore Viability Counts of 90TS Diluted (1:20) with Water (pH 8.0) over a Nine-Day Period.

Days Following Dilution	Average Colony Count 1/
	(10^8)
1	217
2	226
3	215
4	181
8	212
9	213

1/ Average of three plates.

TABLE II

Determination of mortality of dead and dying Malacosoma larvae taken from sprayed and unsprayed areas, Zion Bacillus thuringiensis Control Project, 1965

Dates Larvae		SPRAYED				UNSPRAYED 1/			
		Preserved:				Preserved:			
		70% Alcohol		Dry		70% Alcohol		Dry	
		Mortality Cause		Mortality Cause		Mortality Cause		Mortality Cause	
Collected	Smeared	Bt	Other 2/	Bt	Other 2/	Bt	Other 2/	Bt	Other 2/
		(No.)	(No.)	(No.)	(No.)				
4/30	5/2	5	0	5	0	0	5	0	5
5/3	5/4	5	0	5	0	0	5	0	5
5/5	5/7	4	1	5	0	0	5	0	5
5/7	5/10	1	4	5	0	0	5	0	5
5/10	5/12	0	5	5	0	0	5	0	5
5/12	5/13	1	4	5	0	0	5	0	5

1/ Dead larvae from the unsprayed area were difficult to find. Approximately half were dead; the remaining half appeared moribund and may have eventually died from natural causes.

2/ Cause unknown.